Journal of Labelled Compounds and Radiopharmaceuticals

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16TH WORKSHOP OF THE INTERNATIONAL ISOTOPE SOCIETY–CENTRAL EUROPEAN DIVISION. THE SYNTHESIS AND APPLICATIONS OF ISOTOPES AND ISOTOPICALLY LABELLED COMPOUNDS

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Keywords: Synthesis; carbon-13; carbon-14; deuterium; tritium; imaging; PET; chromatography; environment

ORAL PRESENTATIONS

ISOTOPE INCORPORATION USING ORGANOBORON REAGENTS

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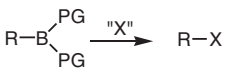
Introduction: The use of organoboranes as precursors to isotopically labeled pharmaceuticals has been of continuous interest in our laboratory for over 25 years.[1] Although boronic acids can be prepared containing a wide variety of functional groups and are generally easier to separate from the radioiodinated products, their use in radiopharmaceutical chemistry has not kept pace with that of other organometallic reagents. The situation has changed dramatically in recent years due to Suzuki/Miyaura chemistry that has made the precursor boronic esters readily available in the laboratory (and commercially.) We have developed a number of organoborane-based reactions that utilize boron reagents as starting materials in the preparation of molecules of use in medicine and agriculture, including tomographic imaging applications.

The presentation will present an overview of organoborane transformations focused on the incorporation of both stable and radioactive isotopes into molecules of utility in pharmaceutical, medicinal, and agricultural applications. The use of novel reaction conditions employing microwave irradiation and solvents such as the ionic liquids will also be discussed. Recently developed methods using triolborate and organotrifluoroborate derivatives will be highlighted. Trifluoroborate salts, for example, have proven to be versatile reagents because of their remarkable chemical reactivity.

Results and Discussion: The utility of the organoboranes rest on the facts that they are essentially non-toxic and often stable to both air and water (with the exception of trialkylboranes). In addition, they are readily prepared and often commercially available which makes them amenable to situations where libraries of active agents must be created. Early studies lead to the realization that a wide variety of elements could replace a boron atom in a regiospecific and stereospecific manner under very mild reaction conditions.



where X = OH, NH_2, CH_2OH, halogen, etc. (PG = halide, OR, etc.)

and R could contain —CO_2H, —C≡N, —SH, NH_2 halogen, etc.

work in our laboratories has focused on developing simple conditions for insertion of isotopically useful 'X' moieties into organic substrates ('R') of value to society. The overall concept is illustrated for biologically important radioisotopes but the corresponding stable isotopes have also been investigated (nitrogen-15, carbon-13, oxygen-17, etc.). The discussion will include historical anecdotes and an overview of our recent studies in our laboratories and the laboratories of other synthetic groups.

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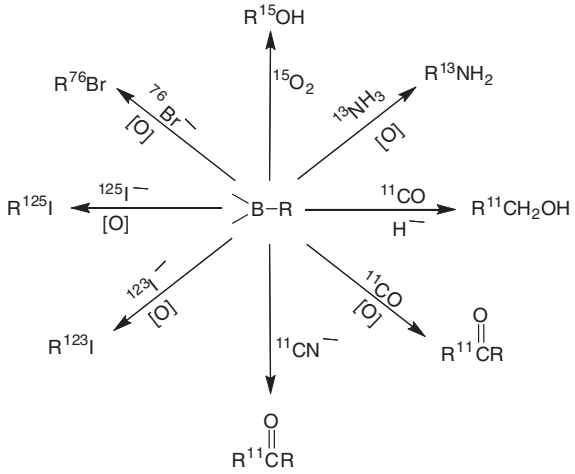


New developments centered on the use of solid state precursors will also be highlighted. These include the use of polymeric trifluoroborate and triolborate reagents for radiohalogenation studies involving the PET and SPECT imaging of amyloid bearing mice, Figure 1.

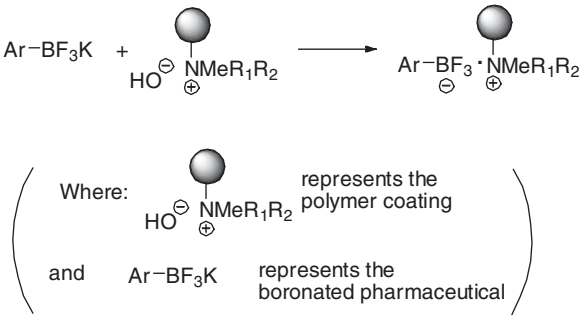
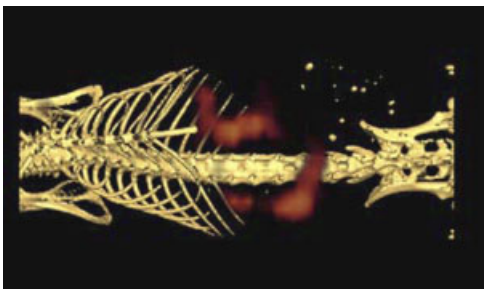


Figure 1. Iodine-123 labelled curcumin study (SPECT/CT).



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TRITIUM LABELLING OF A NEW TUBERCULOSIS DRUG, UNDESIRED ISOTOPE EFFECTS AND ALTERNATIVE SOLUTION VIA ICP-MS

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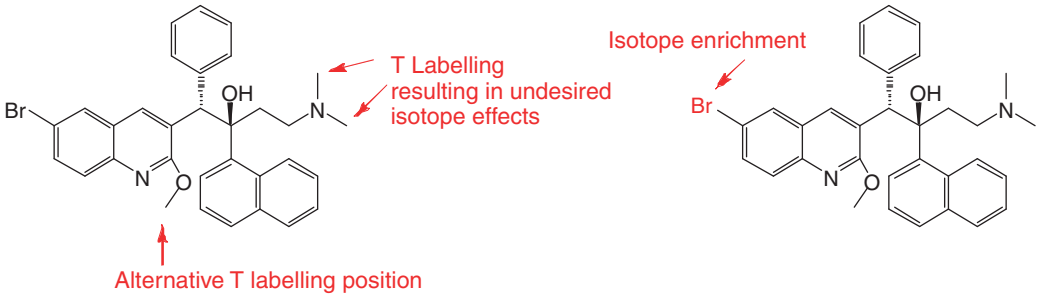
Tuberculosis (TB), which kills two million people annually, is surpassed only by AIDS as the most lethal infectious disease. It is tied inextricably to the AIDS epidemic, disrupting in immune-compromised AIDS patients and often killing them before the AIDS virus does. The last TB-specific drug was discovered in 1968 but newer and better drugs are urgently needed to shorten the duration of TB treatment and to reduce the emergence of drug resistance. In 2005 Johnson and Johnson published results of a very promising drug (R207910 or TMC207) to treat TB based on a novel mechanism of action.[1]

Although pharmacokinetic studies in mice are very promising, profound studies of the compound's properties are required before it can enter into clinical development. To support these studies a tritium labelled compound was synthesized, but an undesired

isotope effect was observed. Due to this, the data generated by using this radiotracer were not reliable and an alternative approach was needed. An Iridium catalysed exchange reaction was investigated and in parallel a new technique was explored using bromo isotope enriched compound and inductively coupled plasma mass spectrometry (ICP-MS), which showed to be a very powerful alternative for radiotracer technology.[2]



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100 YEARS NOBEL PRICE 1908–ERNEST RUTHERFORD (PART II 1909–1937)

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In December 1908 Rutherford received the Nobel Prize (in chemistry!). The background will be discussed.[1]

1908–1911: He continued the work of J.J. Thomson (plum pudding) by counting single alpha particles, establishing his atomic model. 'Now I know, how the atom is looking inside'.[2,3] The number of young and subsequently famous research fellows in his lab was steadily increasing from McGill to Manchaster and Cambridge: F. Soddy, O. Hahn, H. Geiger, E. Marsden, N. Bohr, G. Hevesy, H.G. Moseley, J. Chadwick, J.D. Cockroft, P.L. Kapitza and others.

1915–1919: His experiments of bombarding N with alphas showed the first atomic transmutation.[4] At least a second Nobel Prize should be awarded to him now. Rutherfords famous prediction in the second Bakerian Lecture of 1920, e^+, d and n, was realized by Chadwick in 1934 (the mysterious year). From Beryllium and alphas neutrons could be produced. He did not realize at the time that much higher energies were available from the recently discovered neutron and from the future development of the cyclotron. Rutherfords last (and only wrong) prediction was: not before man will set their feet to the moon, atomic energy will be available to man.

He died on October 19, 1937 from a bacterial infection and his memorial was set in Westminster Hall, close to Isaac Newton.

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SURVEY OF DIFFERENT TRITIUM-LABELLING METHODS USED AT RC TRITEC

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The radiosynthesis group of RC TRITEC is specialized in custom tritium synthesis and therefore has gained a lot of experience in a large variety of different tritium labelling methods. In the talk we present a survey of the projects carried out in the past years focusing on the diverse characteristics and popularities of the different methods.

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All applied labelling reactions have been classified according to the following scheme of five basically different methodologies,[1] which were again split up in subclasses:

H/T exchange:
- T_2, homogeneous catalysis
- T_2, heterogeneous catalysis
- T^+ exchange (acid/base catalyzed)

Tritium gas reductions:
- Halogen/T exchange
- Saturation of C = C/≡CC
- Saturation of C = Q/≡CQ

Tritide reductions:
- Sodium borotritides
- Lithium borotritides
- Aluminium tritides
- Tin tritide

Methylations:
- Methylation of heteroatoms
- C-methylation

Coupling of ^{3}H-intermediates:
- ^{3}H-Ethanol amine
- ^{3}H-Methyl amine
- ^{3}H-Carboxylic acids
- ^{3}H-Amino acids

These method classes are illustrated and discussed with regard to specific activities, regioselective labelling, tritium efficiency, possible labelling candidates and precursors. The varying popularities of the different methods used at RC TRITEC in the past years are statistically analyzed.

Reference

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STRATEGIES FOR ^{3}H-LABELLING OF DECAPEPTIDE HOE 140

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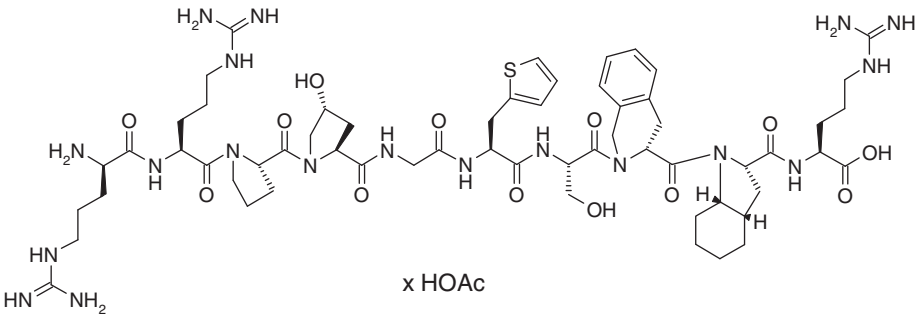
Different strategies for ^{3}H-labeling of decapeptide HOE 140 will be presented. We have evaluated the approach to achieve a maximum specific activity with minimum of hot steps. Neither attempts to introduce the tritium label into the full-length peptide nor the approach to label a single amino acid and to introduce it into the peptide chain were successful. The best way to make the desired compound was to label a large subunit and to build up the complete peptide chain in just a few hot synthetic steps.



HOE 140

THE DETERMINATION OF ISOTOPIC ENRICHMENT BY SUPERCRITICAL FLUID CHROMATOGRAPHY-ELECTROSPRAY IONISATION-MASS SPECTROMETRY USING D3-TESTOSTERONE AS A MODEL COMPOUND

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The level of radioactive isotopic enrichment (specific activity) is one of the key pieces of information that is needed to ensure the quality of any manufactured radiochemical. Typically scintillation counting and spectroscopic techniques, stand alone and coupled with gas and high performance liquid chromatography, are used to determined this value. The safety and change control procedures which govern our operation are designed to create the highest level of confidence in health and safety in any given process. Those involving radioactivity are of particular concern to ensure that the risk of any dose is kept to an absolute minimum.

This paper seeks to demonstrate that supercritical fluid chromatography used in conjunction with mass spectrometry is a viable separation technique that will support the determination of isotopic enrichment by satisfying the following objectives:

- To evaluate the containment of the process.
- To compare the isotope enrichment values determined by SFC and EI+.
- To establish a robust SFC-MS interface.
- To ensure the safety of the system before committing radiolabelled compounds.

5 YEARS OF H/D EXCHANGE AT SANOFI-AVENTIS

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Isotope synthesis departments in the pharmaceutical industry are faced with an increasing demand for a fast supply of stable isotopically labelled new drug development candidates due to the standard use of LC/MS for the analysis of biological samples and the acceleration of development timelines. For the standard preparation besides a conventional synthesis starting from commercially available labelled precursors, a direct labelling approach could be a cost and time efficient alternative if it can be carried out directly on the target molecule or an advanced intermediate. Therefore we have investigated a potential industrial application of H/D exchange for stable isotope labelling. Both recent efforts and experiences in the implementation of H/D exchange and our own method developments will be discussed in context with the published literature and the specific requirements for internal standard applications.

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SYNTHESIS OF ANALOGUES OF PURINE NUCLEOTIDES SELECTIVELY LABELED BY TRITIUM ON THE C-8 OF THE PURINE RING AND EVALUATION OF THE STABILITY OF TRITIUM LABEL

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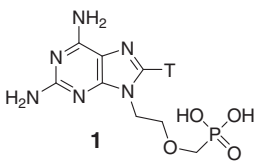
Introduction: Acyclic nucleotide analogues have distinct antiretroviral activity. The high antiviral potency of these compounds is dependent on the rate of their transport across the cell membranes and on the stability inside the cells.[1] Thus in the quest for new target cells for these acyclic nucleotide analogs quick screening of these qualities is desirable.

Results and Discussion: The 9-(2-phosphonomethoxyethyl)-2,6-diamino[8-^{3}H]purine (**1**), 9-(2-phosphonomethoxyethyl)-[8-^{3}H]guanine (**2**) and (R)- 9-(2-phosphonomethoxypropyl)-[8-^{3}H]adenine (tenofovir) (**3**) were prepared by catalytic dehalogenation of corresponding 8-bromo derivatives.



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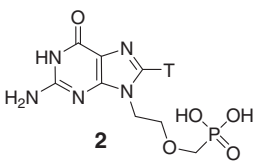

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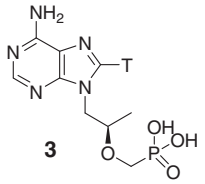

3

The structure of the labeled nucleotide analogs was confirmed by ^{3}H NMR. The stability of the labeled compounds stored in liquid nitrogen was monitored by radio/HPLC. The stability of the label in the neutral water solution at room temperature i.e. under the conditions of biochemical experiment was studied by ^{3}H NMR.

The work was supported by the ASCR project No. Z40550506.

Reference

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STABLE AND RADIOLABELLED SYNTHESES OF THE AGRICULTURAL CHEMICAL FUNGICIDE PROPINEB AND ITS METABOLITES

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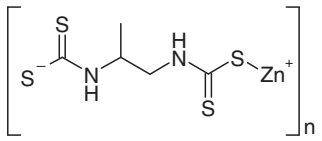
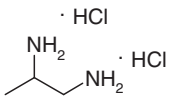
The agricultural chemical fungicide Propineb (polymeric zinc 1,2-propylenebis(dithiocarbamate)), introduced in the market in 1965 by Bayer under the trade name Antracol®, today still provides valuable protection of e.g. grapes, vegetables, tree fruits and potatoes against several economically relevant fungal diseases. In the course of the global (re-)registration of this active ingredient, new studies with stable labelled (^{13}C, ^{15}N) and radiolabelled (^{14}C) parent active ingredient and metabolites were performed that underlined the full compatibility of Propineb with respect to the regulatory requirements concerning human and environmental safety.

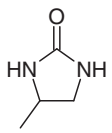
The syntheses of the required stable labelled and radiolabelled parent active ingredient and metabolites (propane-1,2-diamine, propylene urea, propylene thiourea) were performed by the Bayer CropScience Special Synthesis Isotope Chemistry group and will be described in this presentation:



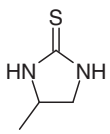
Propineb
labeled: [$^{13}C_5$, $^{15}N_2$] and [propane -1-^{14}C]



propane-1,2-diamine dihydrochloride
labeled: [$^{13}C_3$, 1-^{15}N] and [1-^{14}C]



propylene urea
labeled: [5-^{14}C]



propylene thiourea
labeled: [4-methyl-4,5-$^{13}C_3$, 1-^{15}N] and [5-^{14}C]

Reference

[1] F. Grewe, *Pflanzenschutz-Nachrichten Bayer* **1967**, *20*, 581–599.

TRACING FLUID TRANSPORT PROCESSES IN GEOSYSTEMS ACROSS SCALES

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Introduction: The scientific activities of the department of Georadiochemistry are focusing on the investigation of transport processes in geosystems by means of radiotracer applications. We perform process studies in order to understand fluid dynamics and solute transport in geomatrices by means of radiotracer applications at laboratory scale. Applied methods range from the development of radiotracer substances,[1,2] studies of elementary processes in geochemical systems, including kinetic effects[3,4] (batch and column experiments) and by direct observations of transport processes by means of the tomographic method Geo-PET, which was developed at the IIF in the past recent years.[5–8]

Our activities aim at the quantification of individual processes involved in reactive transport processes and so contribute to the further development of conceptual and numerical models with the final goal of more reliable predictions of risk assessment studies.

Selected results and Discussion

Results will be presented in respect to two scientific fields of expertise:

(A) Adsorption/desorption studies (batch) as a function of the concentrations of two or more dissolved reaction partners, and
(B) Tomographic studies of the concentration distribution of non-reactive solute transport in heterogeneous geologic material.

Immediate aims of these experimental studies are A) the identification and quantification of individual or combined concentration dependent processes (without spatial mapping, 0–dimensional) and B) the identification and quantification of factors inducing material and process dependent spatiotemporal concentration distributions of dissolved non-reactive species in heterogeneous porous media (3–dimensional). Combined A- and B-type studies–that are studies of *reactive transport processes in the heterogeneous media*–imply scaling from the homogeneous system to the heterogeneous macroscopic scale.

Such combined studies will be realised experimentally as means for verification and calibration, once a powerful simulation tool is at hand allowing for the adequate superposition of the previously individually studied processes.

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SPECIFIC [^{14}C]METHYLENE GROUP INTRODUCTION INTO A STEROID VIA OLEFIN CYCLOPROPANATION

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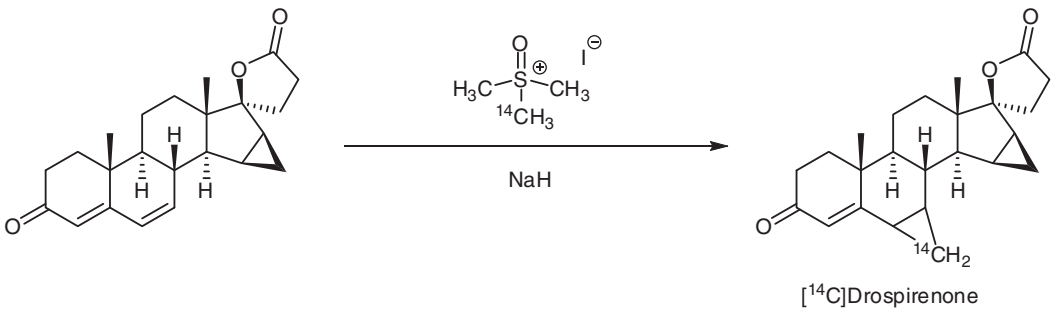
Introduction: For further comprehensive studies on the well-known synthetic progestin Drospirenone, a resynthesis of [^{14}C]Drospirenone was required. Previous C-14 labelling of Drospirenone was accomplished *via* the Corey-Chaykovsky-reaction[1] using [^{14}C]methyl dimethylsulfoxonium iodide and the $\alpha\beta\gamma\delta$-unsaturated carbonyl steroidal precursor (Scheme 1).



Scheme 1. C-14 labelling of Drospirenone.[2]

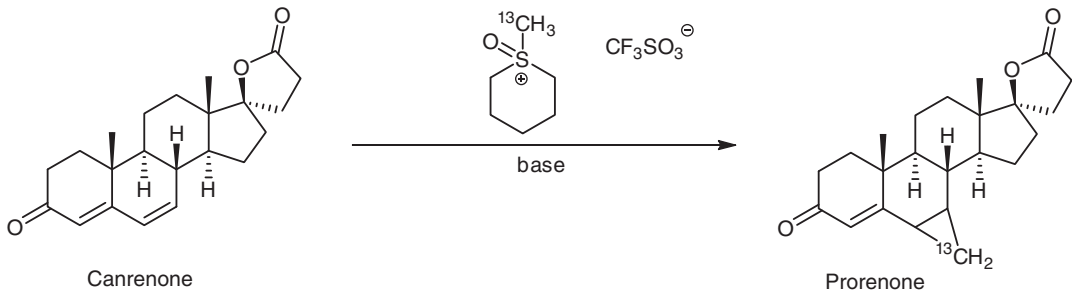
This method unfortunately, has a number of major drawbacks:

1. The [^{14}C]-labelled reagent has three equally reactive methyl groups, of which only one is [^{14}C]-labelled, therefore, the molar specific activity of the product can at best only be one-third compared to the molar specific activity of the [^{14}C]-labelled reagent.
2. The radiochemical yields are poor.

The use of a Corey-Chaykovsky reagent labelled with three [^{14}C]methyl groups would allow a C-14 incorporation with high specific activity, however, the advantage of the high specific activity would be countered by the low radiochemical yield. Furthermore, the synthesis of the [$^{14}C_3$]Corey-Chaykovsky reagent by conventional methods, i.e. reaction of DMSO and methyl iodide, are inefficient giving very poor yields.

For these reasons we embarked upon the search for alternative synthetic methods to introduce C-14 *via* the cyclopropanation reaction to ultimately give [^{14}C]Drospirenone in high specific activity and high radiochemical yield.

Results and Discussion: Canrenone and benzylideneacetophenone (chalcone) were used as readily available model compounds to study various cyclopropanation reactions using unlabeled or stable [^{13}C]-labelled reagents among them diazomethane, nitromethane, and derivatives of the Corey-Chaykovsky reagent. Some reagents, i.e. nitromethane showed good conversion for the $\alpha\beta$-unsaturated carbonyl compound chalcone, but unfortunately did not react with Canrenone.



Scheme 2. Evaluation of cyclopropanation reactions using a model compound.

Finally a reagent, [^{13}C]methyl(pentamethylene)-oxosulfonium triflate, met our expectations. This reagent can be regarded as a derivative of the Corey-Chaykovsky reagent, whereby two of the methyl groups have been replaced by a pentamethylene moiety. After treatment of an equimolar amount of the oxosulfonium triflate (with respect to Canrenone) with sodium hydride, the labelled [^{13}C]methylene group of the resulting ylide was selectively incorporated into Canrenone to give the cyclopropyl ring containing product Prorenone (Scheme 2). The chemical yield was similar to the 'classical' reagent when using stoichiometric amounts. Unexpectedly, no side reactions following a putative deprotonation of the ring methylene groups adjacent to the sulphur were observed.

In addition, the cyclopropanation reagent [^{13}C]methyl(pentamethylene)-oxosulfonium triflate (as well as its ^{14}C or ^{2}H analogues) can be synthesized in excellent yields in three steps, starting from pentamethylene sulphide und methyl iodide. The corresponding sulfonium salt was obtained by replacement of the counterion iodide by triflate; periodate oxidation of this triflate gave the sulfoxonium labelling reagent.

This new variation of the Corey-Chaykovsky cyclopropanation method was successfully applied for the synthesis of [^{14}C]Drospirenone, yielding the product with the desired high specific activity.

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ISOTOPES IN DRUG METABOLISM–SELECTED APPLICATIONS FOR ISOTOPE-LABELED COMPOUNDS

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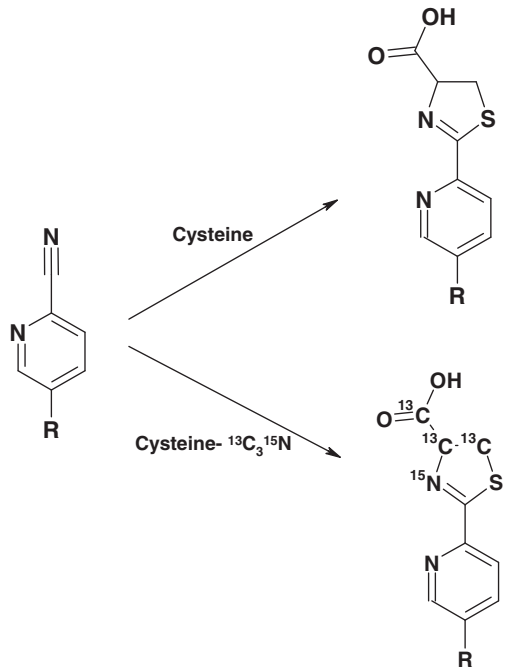
Introduction: Metabolism studies are essential in the drug discovery and development processes and are important to support the safety studies and the clinical development of drug candidates. These include studies on identification and structure elucidation of metabolites, drug-drug interactions, mechanisms of metabolite formation and identification of enzymes involved in the metabolism.

Results and Discussion: Examples will be given for selected applications of compounds labelled with radioisotopes or stable isotopes for different metabolism studies. Isotope dilution in combination with high resolution mass spectrometry is a very powerful tool especially for metabolite identification and the elucidation of metabolite structures as well as metabolic reaction mechanisms (Scheme 1). Furthermore stable labelled drug substances or metabolites, usually synthesized for quantification reasons can also be used for structure elucidation of metabolites. Simple reagents, as e.g. $H_2^{18}O$, can be used to differentiate enzymes catalyzing very similar hydroxylation reactions.



Scheme 1.

RAMONA-QUATTRO: IMPROVED SENSITIVITY OF A RADIOACTIVITY-HPLC-FLOW-DETECTOR

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Introduction: 14C-Radioactivity-HPLC-Flow-Detectors have been used since 1970 for simultaneous on-line recording with UV- and other flow detectors in liquid chromatography. The demand for a detector suitable for lower flow rates and higher sensitivity has grown considerably. Dederichs et al [1] have introduced a new 14C-radioactivity-μ-HPLC-Flow-Detector, which offers higher sensitivity at lower flow rates. This development principle was extended to higher sensitivity at usual flow rates.

Basic facts: The sensitivity–the number of counts from a certain nuclide and activity sample per time–depends in flow applications on the efficiency of the scintillator, but mainly on the counting time in a flow cell. Larger flow cells collect at the same flow-rate more counts, but too large flow cells damage the chromatographic resolution. The rule of experience indicates, that the cell-volume should be 1/5 to 1/10 of the peak-volume.

Small flow rates of μ-HPLC produce small peak volumes and therefore require small flow cells and a very small dead volume. Smaller flow cells count a smaller number of counts and the resulting sensitivity is small. 4 flow cells after each other count a chromatogram 4 times and that improves the sensitivity by a factor of 4. Close to the background the sensitivity improvement may be reduced to a factor of 2.

Development: 4 radioactivity-coincidence-flow-detectors are assembled as close as possible together, in order to make the flow time from one detector to the following as small as possible. The individual flow cells of each detector are designed in one block, one cell next to the following as close as possible, but the potential 'spill-over' from one flow detector to the next has to be avoided completely. 4 radiochromatograms are recorded individually. The radiochromatogram pattern of the first detector to the second, the third and the fourth is delayed by the time the eluate requires to flow from the one to the following detector. The flow times from the first detector to the second, third and fourth are determined individually and subtracted from the following chromatogram flow-times. This presents all chromatograms on the same time scale. Now the counted events of each interval in all 4 chromatograms are added up to a sum-chromatogram. In case the individual flow time from one chromatogram record is determined exactly and subtracted from the individual run time correctly, the sum chromatogram shows exactly the same chromatogram pattern. Now the counts in every interval are the sum of the corresponding intervals of all single chromatograms.

Discussion: The chromatogram pattern of every single flow detector was analysed carefully. There are potential changes in the peak shape of the chromatogram possible depending whether solid scintillator or liquid scintillator admixture at certain flow rates were applied. A small tendency of a certain peak-broadening was obtained from the first to the last flow detector. Measures were taken in order to avoid peak-broadening and a sum-chromatogram was obtained with almost no peak broadening.

At μ-HPLC applications small flow cells with small photomultiplier were applied. Small photomultiplier can produce less background counts. In an 4×4 photomultiplier arrangement the low background countrate does not add up linearly but squared under the square root. That improves the sensitivity considerably.

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At conventional HPLC applications with flow rates of typical 1ml/min, photomultipliers with conventional photocathode sizes were used.

The sensitivity of Ramona quattro was compared to Ramona ∗ and the improvement of minimum factor 2 was confirmed. Results will be demonstrated.

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HANDLING OF TRITUM REAGENTS IN MULTI-CURIE SCALE

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State-of-the-art tritium-labelling techniques require the application of tailor-made tritium-labelled reagents, which have to be synthesized according to radiation protection rules. Therefore, the high vacuum manifold system completely developed and manufactured by RC TRITEC AG is used. In the talk the applications of different labelling reagents synthesized at the stainless steel manifold system are presented.

These custom-made manifolds (Fig. 1) are used at RC TRITEC AG to handle multi-Curies of activities for the preparation and application of methyl iodide (CT_3I), tritiated water (HTO) and a huge variety of tritide derivatives. Methyl iodide is produced in batches of up to 10 Ci with specific activities of 60–80 Ci/mmol. Amongst the typical methylation[1] of amine and phenol derivatives, Stille cross coupling (Fig. 2) with CT_3I is presented. Tritiated water is produced from PtO_2 and tritium gas in quantities of 10 to 20 Ci. The handling of HTO in large quantities of activity requires special precautions, but facilitates regioselective labelling under pH dependent catalysis[2] (Fig. 3). Finally, a versatile precursor for tritide reagents is LiT, which can be converted to a variety of tritide reagents[3] with different chemoselectivities (Fig. 4).

The experimental set-up avoiding any atmospheric release of highly volatile labelled reagents or by-products is described.

Figure 1. Manifold system.

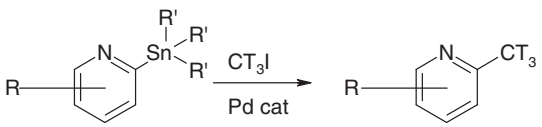

Figure 2. Stille coupling using CT_3I.

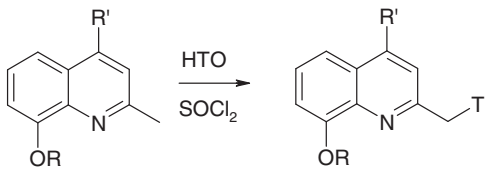

Figure 3. Acid catalyzed exchange with HTO.

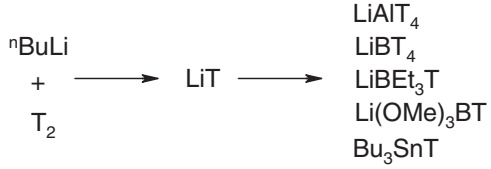

Figure 4. Lithium tritide as a fundamental precursor for chemoselective tritides.

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PRACTICAL USE OF RADIO-LABELLED COMPOUNDS IN TESTING FOR ENVIRONMENTAL RISK ASSESSMENT

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Beside many different chemical, physical and medicinal applications, radio-labelled compounds also have their important uses in environmental research. Some of the current OECD guideline environmental fate and bioaccumulation assays are presented, with their requirements (also for the test substance respectively the best position for radio-labels), test set-ups, examples of results and interpretations.

NEW BISPIDONE TYPE LIGANDS AND THEIR APPLICATIONS

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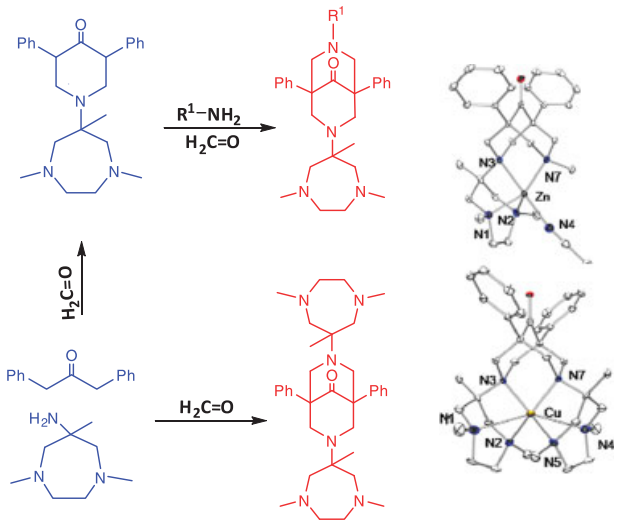
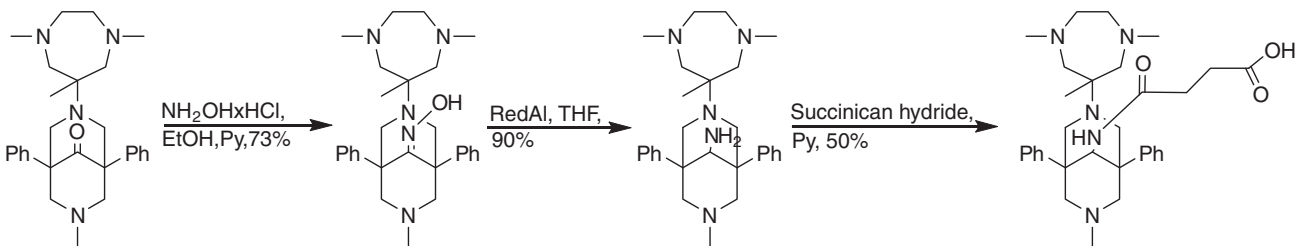
Bispidones are rigid bicyclic ligands with variable donor sets. Due to their add-on system synthesis, they can be adopted to a desired size of ligand cavity. Already known with octahedral coordination geometry,[1], this new system favors a distorted trigonal-bipyramidal to trigonal-prismatic coordination environment[2].



The Bispidones presented here show a high selectivity for Cu^{II}. Potentiometric titrations of $\log_K$ revealed values from $\log_K = 19.5$ to cyclam like $\log_K = 26.4$. Complex stabilities for Co^{II}, Ni^{II} are less stable. According to their copper complex stability, these ligands can be used as labelled ^{64}Cu-(Diagnosis/PET/SPECT)- and/or ^{67}Cu-(Therapy)-complex system. Functionalisation has been added to create a docking point for bioconjugates like peptides or antibodies.[3,4]



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CHALLENGES IN THE DEVELOPMENT OF SGLT INHIBITORS AVE2268 AND AVE8887

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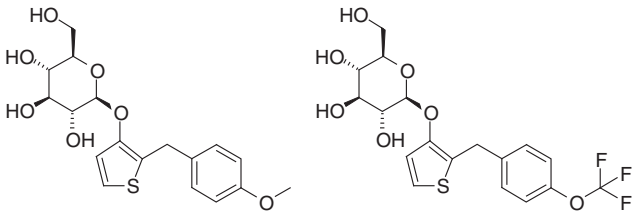
AVE2268 and AVE8887 are developed as novel inhibitors of sodium dependent co-transporters (SGLT-2) that can transport glucose across cellular membranes against a concentration gradient. In animal models they increased renal excretion of glucose. This area is a field of intensive pharmaceutical research which is demonstrated by around 60 patents filed in the last six years. Both compounds were developed in the indication type II diabetes mellitus and AVE2268 is actually in clinical development.



AVE2268 **AVE8887**

We describe in detail the challenges of the synthesis of isotopically labelled compounds of SGLT inhibitors **AVE2268** and **AVE8887**. Although very similar in their chemical structure different synthetic pathways had to be developed.

Furthermore we will show in the synthesis of two of their metabolites unexpected synthetic challenges and possible new strategies to overcome them.

1-^{13}C-OCTANOIC ACID PREPARATION FOR MEDICINE DIAGNOSTICS

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1-^{13}C-Octanoic acid is medicinal substance used for the diagnosis of stomach empting [1] and also for syntheses of a number of derivatives which are used for diagnosis of other diseases. The required isotope enrichment of 1–^{13}C–octanoic acid is 98–99%.

In our opinion, 1-heptene hydrocarboxylation with carbon monoxide ^{13}CO in presence of catalytic complex $PdCl_2(PPh_3)_2$ is the most convenient method for isotope ^{13}C incorporation into position 1 of the acid molecule (carboxylic group) among the known methods of octanoic acid preparation [2]:

$$C_5H_{11} - CH = CH_2 + {}^{13}CO + H_2O \rightarrow C_7H_{15} - {}^{13}CO_2H.$$

The reaction is conducted at a temperature of 150°Ñ and a pressure of 3–5 bar; ^{13}CO isotope purity is 99.8%. 1-^{13}C-Octanoic acid yield is 97%, and isolated product contains 99.2% of base material and 0.8% of 1-^{13}C-2-methyl heptanoic acid as a mixture. The isotope purity of the final product is determined using ^{13}C-NMR spectroscopy. In the spectrum of the prepared acid α-carbon atom (C-2) signal is a superposition of two signals present: doublet (34.035 ppm, $^1J_{C-C} = 55.08$ Hz) of labelled octanoic acid fragment HO-^{13}C(O)-^{13}CH$_2$- and singlet (34.048 ppm) of unlabelled acid fragment HO-^{12}C(O)-^{13}CH$_2$-. The ratio of signal intensities of labelled acid and unlabelled allows the determination of the 1-^{13}C-octanoic acid content in the mixture.

It has been proved that the preparation of 1-^{13}C-octanoic acid, having high isotope purity by the method under consideration, is possible only in the absence of unlabelled carboxylic acid or other compounds which can decompose with unlabelled CO formation under olefin hydrocarboxylation conditions [3]. Therefore, using propionic acid and *o*-xylene mixture as a solvent, the isotope purity of the prepared 1-^{13}C-octanoic acid was only 80%. However in the absence of propionic acid (*o*-xylene the only component of solvent) the isotope purity of 1-^{13}C-octanoic acid was 98.3%.

Using 2D spectra COSY and HSQC we have also revealed the other sequence of octanoic acid ÑÍ$_2$-group signals in ^{13}C-NMR spectrum in comparison with the sequence consisted in the known spectral base SDBS [4].

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^{13}C-UREA SYNTHESIS BY OXIDATIVE CARBONYLATION OF AMMONIA

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^{13}C-Urea is widely used for the urease Breath test diagnosis of Helicobacter pylori. The well known methods of urea preparation are characterized by a number of drawbacks. The traditional method of urea industrial production, based on CO_2 uses very rigorous conditions (200°Ñ, 250 bar).[1] So called 'cyanide' methods[2] use rather toxic compounds and are multistage, leading to considerable losses of isotope label.

It is known however[3,4] that urea can be prepared by reaction of CO with NH_3 in the presence of selenium compounds. Using stoichiometric quantities of Se the process proceeds under very mild conditions (r.t., 1 bar);[3] in the presence of SeO_2 oxygen addition provides a catalytic process,[4] however catalyst activity is not high–52.7 h^{-1} (30°Ñ, 40 bar).

We have developed a new one-stage effective method of ^{13}C-urea preparation by the reaction of carbon monoxide ^{13}CO with ammonia. The reaction proceeds under sufficiently mild conditions (40°Ñ, 14 bar CO) in the presence of selenium. Oxygen is added to the reaction gas mixture to ensure a catalytic process:

$$^{13}CO + 2NH_3 + 1/2\,O_2 \rightarrow (NH_2)_2{}^{13}CO + H_2O.$$

The process is conducted in a mixture of THF and methanol. Urea is formed as the only product without any by-product (mp 131–133°Ñ; í, sm^{-1}: 3494 m., 3389 m., 1691 s., 1610 s., 1030 v.s., 919 s.; HPLC/ESI-MS). The reaction rate is very high: the catalyst turnover number (TON) achieves 155 for 20 min and then process stops, giving specific catalyst activity (turnover frequency) TOF = 465 h^{-1}. The product yield calculated on the loaded CO is 86.5% and the urea yield may be raised to 92.4% by an additional supply of oxygen. Residual of Se does not exceed 2.2×10^{-3}%. The influence of CO/O_2 ratio and solvent composition was studied. Isotope purity of urea prepared (isotope ratio mass spectrometry method using isotope dilution) is not less than 96% and will be increased in the future.

So, the base of the effective liquid phase catalytic process of ^{13}C-urea preparation from ^{13}CO and ammonia in mild conditions has been developed.

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^{13}CO PREPARATION FROM INDUSTRIALLY AVAILABLE $^{13}CO_2$

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Carbonylation reactions based on carbon monoxide (CO) are amongst the most effective methods of carbon isotope label introduction into organic molecules.[1–3] Carbon dioxide reduction by metal zinc is used for CO preparation.[4,5] However the known methods are often characterized by small product output due to low CO yields and apparatus construction. Moreover, zinc powders may eventually sinter under process temperature and that additionally complicates CO production.

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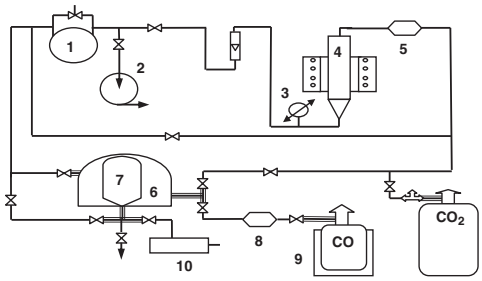
We have developed high capacity ^{13}CO production method[6] for creation in Russia a broad range of ^{13}C labelled organic products. This method is based on high quality labelled carbon dioxide ($^{13}CO_2$) which is produced by home industry with the chemical and isotope purity of more than 99%.

Our method is also based on the reaction $^{13}CO_2 + Zn \rightarrow {}^{13}CO + ZnO$, but modified as described: replacement of zinc by a brass powder precludes sintering during the process and using flowing-circulation installation (Fig. 1) provides the almost quantitative ^{13}CO yield.



1 – Circulating pump
2 – Vacuum pump
3 – Pressure and vacuum gage
4 – Reactor with a furnace
5 – Filter
6 – Gasholder
7 – Elastic chamber
8 – Adsorber
9 – Dewar flask
10 – Compressor

Figure 1. The ^{13}CO production installation scheme. 1 – Circulating pump 2 – Vacuum pump 3 – Pressure and vacuum gage 4 – Reactor with a furnace 5 – Filter 6 – Gasholder 7 – Elastic chamber 8 – Adsorber 9 – Dewar flask 10 – Compressor.

In the case of brass powder marked PR L63 (40–100 μm, 37% of Zn) the installation specific capacity calculated on 1 kg of brass is 1.4 l/h. The process is conducted at reactor heating from 380 to 750°C with the rate of 1.5 grad./min under pressure of 1.2 bars. ^{13}CO yield reaches 98.5% calculated on the loaded $^{13}CO_2$. Residual $^{13}CO_2$ is taken up by zeolite in adsorber down to content of 0.4%. This method allows producing up to 250 liters of ^{13}CO per month. The obtained gas is pressurized into high pressure cylinders using compressor or by condensation under liquid nitrogen temperature (Fig. 1).

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TYROSINE CONTAINING PEPTIDE RESISTENT TO RADIIODINATION. WHAT IS BEHIND?

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Last year at the Bad Soden workshop we mentioned the unsuccessful attempts to label the peptide with following amino acid sequence:

Met-Ala-Pro-Arg-Gly-Tyr-Ser-Cys-Leu-Leu-Leu-Leu-Thr-Gly-Glu-Ile-Asp-Leu-Pro-Val-Lys-Arg-Arg-Ala

One of the proposed explanations of the observed resistance of the peptide tyrosine moiety to undergo the aromatic electrophilic substitution was formation of hydrophobic core which would shield the tyrosine moiety from reaction milieu. The conformation of the phenylalanine and tyrosine containing peptides in water solution was studied by NMR techniques and the results will be presented.

The work was supported by the ASCR project No. Z40550506.

